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(54) Title: LACCASES, NUCLEIC ACIDS ENCODING THEM AND METHODS FOR MAKING AND USING THEM

(57) Abstract: The invention provides laccases, polynucleotides encoding these enzymes, the use of such polynucleotides and polypeptides. In one aspect, the invention relates to the enzymatic production of nootkatone by way of the conversion of valencene using proteins having a laccase activity, e.g., a novel laccase of the invention. In one aspect, the invention provides methods of depolymerizing lignin, e.g., in a pulp or paper manufacturing process, using a polypeptide of the invention. In another aspect, the invention provides methods for oxidizing products that can be mediators of laccase-catalyzed oxidation reactions, e.g., 2,2-azino-bis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS), 1-hydroxybenzotriazole (HBT), 2,2,6,6-tetramethylpiperidin-1-yloxy (TEMPO), dimethoxyphenol, and the like.

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A. CLASSIFICATION OF SUBJECT MATTER IPC: C07H 21/04(2006.01);C12Q 1/68(2006.01);G01N 33/53(2006.01);C12P 19/34(2006.01);C12N 1/21(2006.01);5/02(2006.01);9/02(2006.01);15/00(2006.01);15/05(2006.01);15/53(2006.01);15/63(2006.01);15/82(2006.01) USPC: 536/23.2,24.32,24.33;435/6,7.1,91.2,189,252.3,320.1,410,440 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 536/23.2, 24.32, 24.33; 435/6, 7.1, 91.2, 189, 252.3, 320.1, 410, 440 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KASAHARA et al. Sequence Analysis of the groESL-cotA Region of the Bacillus Subtilis Genome, Containing the Restriction/Modification System Genes. DNA Research. October 1997, Vol. 4. No. 5, pages 335-339. The gene encoding gutB, dehydrogenase, is 77.3% identical to SEQ ID NO:1.	1-4, 6-25, 27-37, 50-54, 56, 57, 59, 106, 107, 110, 128, 130-150, 158-161, 168-172, 197-199, 259-265, SEQ ID
A	US 6,015,783 A (VON DER OSTEN et al) 18 January 2000 (18.01.2000). The gene encoding Myceliophthora thermophila laccase is 27.2% identical to SEQ ID NO:23.	1-37, 39-47, 50-54, 56, 57, 59, 106, 107, 110, 128, 130-150, 158-161, 168-172, 197-199, 259-265, SEQ ID NO:23.
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents:		
"A"	document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed	
Date of the actual completion of the international search 17 August 2006 (17.08.2006)		Date of mailing of the international search report 13 SEP 2006
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201		Authorized officer Elizabeth Slobodyansky, PhD <i>Janice Ford</i> Telephone No. 571-272-1600 <i>for</i>

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 55,58,190,214 and 216
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Please See Continuation Sheet
3. ☒ Claims Nos.: 126, 127, 129, 151-157, 167, 173, 174, 188, 190
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
 2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of any additional fees.
 3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: Please See Continuation Sheet
 4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
- Remark on Protest
- | | |
|--------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <input type="checkbox"/> | The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee. |
| <input type="checkbox"/> | The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation. |
| <input type="checkbox"/> | No protest accompanied the payment of additional search fees. |

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Continuation of Box II Reason 2:

Claim 55 is drawn to oligonucleotide of claim 49 whereas claim 49 is drawn to an animal. Claim 58 is drawn to RNA of claim 52 whereas claim 52 is drawn to a seed. Claim 214 depends from itself. Claim 216 is drawn to a dairy product of claim 213 whereas claim 213 is drawn to a method.

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I-XIII, claim(s) 1-47, 50-59, 106, 107, 110, 126-155, 158-161, 168-172, 197-199, 259-265, drawn to a nucleic acid having at least 50% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23 or SEQ ID NO:25, respectively, an antisense sequence and various methods of use thereof.

Groups XIV-XXVI, claim(s) 48 and 49, drawn to a transgenic animal comprising any of the above sequences.

Groups XXVII-XXXIX, claim(s) 60-99, 108, 109, 111-115, 162-165, 175-187, 189, 200-213, 215, 217-252, 254-258, drawn to a polypeptide having at least 50% identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24 or SEQ ID NO:26, respectively, a signal fragment thereof, food products comprising thereof and various methods of use thereof.

Groups XL-LII, claim(s) 100-105, drawn to an antibody against any of the above polypeptides, methods of making and use thereof.

Group LIII-LXV, claim(s) 116-125, drawn to a computer system and methods of use thereof.

Group LXVI, claim(s) 253, drawn to a nootkatone.

The inventions listed as Groups I-VI do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: According to PCT Rule 13.2 and to the guidelines in Section (f)(i)(A) of Annex B of the PCT Administrative Instructions, all alternatives of a Markush Group must have a common property or activity. The nucleic acids of Groups I-XIII lack common structure, the animals of Groups XIV-XXVI lack common structure, the polypeptides of Groups XXVII-XLI lack common structure, the antibody of Groups XLII-LV lack common structure and thus, the molecules and animals comprising them share no special technical feature. A nootkatone of Group LXX lacks common structure with nucleic acids, polypeptides, antibodies and animals. The computer systems of Groups LVI-LXIX lack common property or activity with either molecules or animals.

The nucleic acids of Groups I-XIII lack common structure, the animals of Groups XIV-XXVI lack common structure, the polypeptides of Groups XXVII-XXXIX lack common structure, the antibody of Groups XL-LII lack common structure and thus, the molecules and animals comprising them share no special technical feature as the nucleic acid of SEQ ID NO:1, for example, is not required for the polypeptides of SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24 or 26 because SEQ ID NO:1 does not encode a polypeptide having any of these sequences. A polypeptide of SEQ ID NO:2 does not elicit the antibody against a polypeptide having any of the above sequences.

According to PCT Rule 13.2, unity of invention exists only when the shared same or corresponding technical feature is a contribution over the prior art. The inventions of Groups I-LXVI do not relate to a single general inventive concept because they lack the same or corresponding special technical feature. The technical feature of Groups I-XIII is a nucleic acid, which is shown by Kasahara et al (1997)

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DNA Research, 4, 335-339, GenBank accession AB007638, to lack novelty or inventive step because it has at least 77.4% identity to SEQ ID NO:15. Furthermore, the technical feature of Groups XXVII-XXXIX which is shown by Borriss et al (1996) *Microbiology*, 142, 3027-3031, to lack novelty or inventive step because it has at least 94.6% identity to SEQ ID NO:16 (see attached sequence comparisons). Therefore, neither nucleic acids, nor polypeptides make a contribution over the prior art.

Continuation of Box III Item 3:

1-37, 39-47, 50-54, 56, 57, 59, 106, 107, 110, 128, 130-150, 158-161, 168-172, 197-199, 259-265, SEQ ID NOs: 1, 23